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Calcium-Ion Efflux from Brain Tissue Power-Density Versus Internal Field-Intensity Dependencies at 50-MHz RF Radiation

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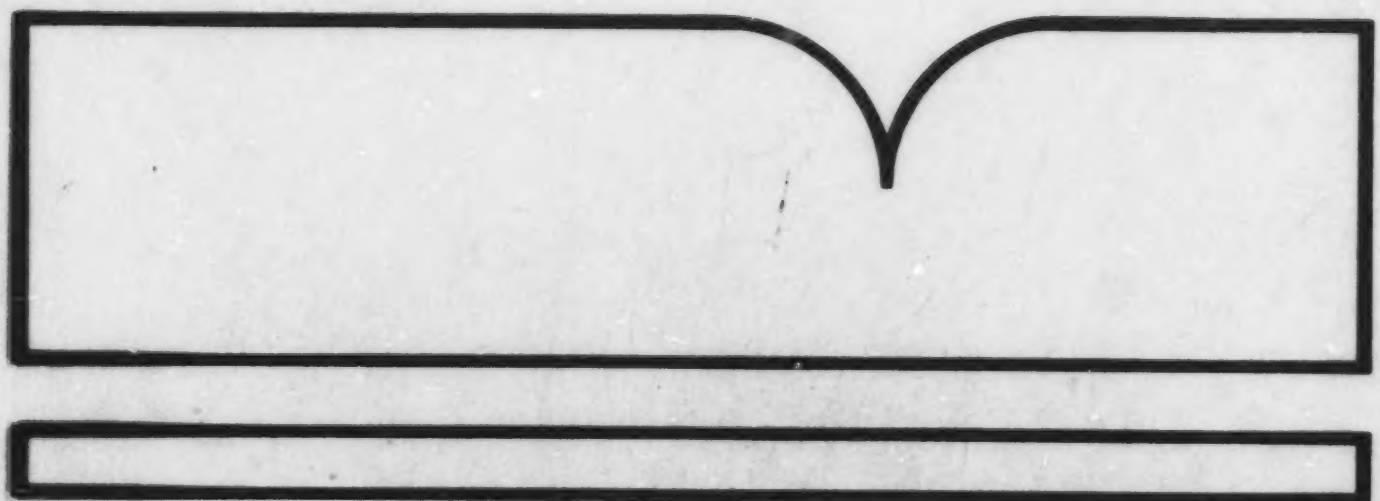

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16. ABSTRACT

In previous experiments changes were found in calcium-ion efflux from chick-brain tissue that had been exposed in vitro to 147-MHz radiation across a specific range of power densities when the field was amplitude modulated at 16 Hz. In the present study, 50-MHz radiation, similarly modulated as a sinusoid, was found to produce changes in calcium-ion efflux from chick brains exposed in vitro in a Crawford cell. Exposure conditions were optimized to broaden any power-density window and to enhance the opportunity to detect changes in the calcium-ion efflux. The results of a power-density series demonstrated two effective ranges: One spanning a range from 1.44 to 1.67 mW/cm^2 and the other including 3.64 mW/cm^2, which were bracketed by no-effect results at 0.72, 2.17, and 4.32 mW/cm^2. Peaks of positive findings are associated with near-identical rates of energy absorption: 1.4μW/g at 147 MHz, and 1.3μW/g at 50 MHz, which indicates that the enhanced-efflux phenomenon is more dependent on the intensity of fields in the brain that on the power density of incident radiation. In addition, the phenomenon appears to occur at multiples of some, as yet unknown, rate of radiofrequency (RF) energy absorption. Because of the extremely small increments of temperature associated with positive findings (<4 X 10^{-4}°C), and the existence of more than one productive absorption rate, a solely thermal explanation appears extremely unlikely.

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Calcium-Ion Efflux From Brain Tissue: Power-Density Versus Internal Field-Intensity Dependencies at 50-MHz RF Radiation

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In previous experiments changes were found in calcium-ion efflux from chick-brain tissue that had been exposed in vitro to 147-MHz radiation across a specific range of power densities when the field was amplitude modulated at 16 Hz. In the present study, 50-MHz radiation, similarly modulated as a sinusoid, was found to produce changes in calcium-ion efflux from chick brains exposed in vitro in a Crawford cell. Exposure conditions were optimized to broaden any power-density window and to enhance the opportunity to detect changes in the calcium-ion efflux. The results of a power-density series demonstrated two effective ranges: One spanning a range from 1.44 to 1.67 mW/cm^2, and the other including 3.64 mW/cm^2, which were bracketed by no-effect results at 0.72, 2.17, and 4.32 mW/cm^2. Peaks of positive findings are associated with near-identical rates of energy absorption: 1.4 $\mu W/g$ at 147 MHz, and 1.3 $\mu W/g$ at 50 MHz, which indicates that the enhanced-efflux phenomenon is more dependent on the intensity of fields in the brain than on the power density of incident radiation. In addition, the phenomenon appears to occur at multiples of some, as yet unknown, rate of radiofrequency (RF) energy absorption. Because of the extremely small increments of temperature associated with positive findings ($< 4 \times 10^{-4}$°C), and the existence of more than one productive absorption rate, a solely thermal explanation appears extremely unlikely.

Key words: calcium ions, brain tissue, radiofrequency (RF) radiation, amplitude modulation, power-density window, 16-Hz modulation, 50 MHz

INTRODUCTION

In previous studies, we reported that only certain power densities of a 147-MHz carrier wave, sinusoidally amplitude modulated between 9 and 16 Hz, can enhance the efflux of calcium ions from chick-brain tissue exposed in vitro [Blackman et al, 1979,

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1980]. The effective power densities ranged between 0.55 and 1.38 mW/cm^2. In similar experiments using a 450-MHz carrier, Bawin et al [1977] reported enhanced efflux at 0.1 and at 1.0 mW/cm^2, in agreement with our findings.

Due to the similar range of effective power densities for 147- and 450-MHz carrier waves, we conducted further experiments to determine if the range of effective power densities exhibits a dependence on carrier frequency. The experiments were performed at a carrier frequency of 50 MHz, using an experimental design that paired the results of each exposure with a companion set exposed at zero power density [Blackman et al, 1980].

MATERIALS AND METHODS

Exposure System

A rectangular transmission line (Crawford cell) was used to expose samples of brain tissue [Weil, 1978; Blackman et al, 1979]. The 50-MHz radiofrequency (RF) carrier was supplied by a crystal-controlled signal generator (Wavetek, Model 3000). The voltage of the carrier was amplitude modulated (> 95%) by a 16-Hz sine wave from a function generator (Kron-Hite, Model 5200). The modulated signal was then amplified by a 9.5-W linear-power amplifier (Electronic Navigation Industries, Model 510L). Forward, reflected, and transmitted powers were monitored by two 20-db bidirectional couplers (NARDA Model 3020A) and three identical thermoelectric power meters (Hewlett-Packard, Model 435A) in order to characterize the distribution of energy within the exposure system. The signal was terminated in a 40-W load (NARDA, Model 376 NM).

A foamed polystyrene chamber housed the transmission line. Air temperature within the chamber was maintained at 37 ± 0.2 °C by means of a proportional temperature controller [Ali, 1975]. Control samples were placed on shelves within the chamber adjacent to the transmission line and were essentially in the same thermal environment as samples in the transmission line.

Materials

The brain tissues were from one- to seven-day-old chicks (Gallus domesticus, Young strain) that were obtained from North Carolina State University.

Media

The medium used in this study was identical to that described in Blackman et al [1980]. It was composed of 155 mM NaCl, 5.6 mM KCl, 2.16 mM $CaCl_2$, 2.4 mM $NaHCO_3$, and 11.1 mM glucose. For the radio-labeling of tissues, the medium was supplemented with $^{45}Ca^{++}$ at 4.6 μCi/ml (New England Nuclear, 2.1 mCi/mmole). All solutions were prepared on the same day that they were used. The pH of the solutions was between 7.6 and 7.8 and the temperature was 37 ± 0.3 °C.

Preparation of Tissues

The chicks were killed by decapitation. The top of the skull and then the forebrain was removed from each chick. Each forebrain was divided at the midline and the two hemispheres were then placed in a test tube. The two hemispheres of each forebrain were maintained as an exposure-control pair throughout subsequent treatments and analyses.

After four brain-tissue pairs were prepared, 1 ml of calcium-45–labeled medium was added to each sample tube; all tubes were then placed in a water bath and agitated for 30 minutes at 37 °C. Following the 30-minute labeling period, the radioactive solution was aspirated and the tissues were rinsed in the following manner: Two milliliters of unlabeled medium was added to each tube and then poured with the tissues into small plastic sieves held in a rack. The tissues were then rinsed by immersion in two 250-ml volumes of unlabeled medium. The rinsing procedure took approximately four minutes. Each tissue, free of any loosely associated radioactive calcium, was then placed in individual polystyrene tubes (17 × 40 mm) which contained 1 ml of medium.

Exposure of Tissues

Polycarbonate racks that held the tissue samples were placed on either side of the transmission line's center conductor. Similar racks containing the control tissues were placed in the foamed polystyrene chamber on shelves alongside the transmission line at a position where the electric-field strength was more than 30 db below the electric field inside the transmission line. Four chick brains were treated at once, with half of each brain used as a control and the other half as an exposed sample.

Four additional brains were treated as described above but without microwave radiation. In this group, the samples placed within the transmission line were subjected to a sham exposure.

This combined series of eight pairs of brains was prepared and treated within one hour and represented one replication of the basic experiment. Several replications were done at each power density. For some power densities, several replications were done together and then several more were done at a later time; this delay introduced a possible latency variable, which was considered in the final analysis.

The tissues were exposed for 20 minutes to 50-MHz radiation in a series of experiments under 16-Hz sinusoidal amplitude modulation; power densities of 0.37, 0.72, 1.44, 1.67, 2.17, 3.64 or 4.32 mW/cm^2 were used. Ten tubes were in the chamber at a time; four tubes contained brain tissue and were placed two on either side of the center plane. Six additional tubes contained only an equivalent amount of unlabeled medium (1.25 ml) and were placed on either side of the sample tubes [cf Blackman et al, 1980; Joines et al, 1980a,b].

Assay for Calcium-Ion Efflux

To estimate the quantity of calcium ions that had been released by each sample of tissue during the period of treatment, 0.2-ml aliquots of bathing solution were added to 5 ml of scintillation cocktail (Amersham/Searles, ACS), and counted in a liquid scintillation counter.

Analysis of Data

The relative quantity of calcium ions released by each pair of hemispheres was defined as the ratio of counts per minute in the treated/control and sham/control paired samples (Vt/Vc).

The data for 1.67 mW/cm^2 was analyzed for the possible effect of time as well as replication. An analysis of variance for a partially nested design was used to test for the time effect ($F\ [1, 14] = 0.77$, $P = 0.395$) and for the replication effect ($F\ [14, 110] = 0.89$, $P = 0.572$). Because no influence of time was found, a two-way analysis of variance was used to test the data at each power density for a replication effect (Table 1). Since

reliable time and replication effects were not found, a one-way analysis of variance was performed for the exposure–sham-exposure combination at each power density. In this analysis, additional data could be included for those combinations that had incomplete replications, which could not be easily included in the analyses of variance for the partially nested and two-way designs.

RESULTS

Table 2 gives the number of sample (n), mean, and standard error of the mean (SE) of Vt/Vc for the sham and exposed groups at each power density. The P value from the one-way analysis of variance is given in the last column of the Table. There was a significant ($P < 0.02$) increase in the mean Vt/Vc of the exposed group as compared to the sham group at three power densities – 1.44, 1.67, and 3.64 mW/cm^2. No effect was observed at 0.37, 0.72, 2.17, or 4.32 mW/cm^2.

Figure 1 is a bar graph of the same data which shows the mean Vt/Vc as a function of incident power density. The number of samples treated at each condition is shown at the top of the SE bars.

TABLE 1. Two-Way Analysis of Variance to Test for a Replication Effect

Power density (mW/cm^2)	Degrees of freedom	F	P
0.37	7,48	0.31	0.945
0.72	11,70	0.43	0.936
1.44	7,48	0.44	0.869
1.67	14,112	0.88	0.580
2.17	8,54	1.64	0.135
3.64	9,108	1.10	0.372
4.32	6,42	0.88	0.518

TABLE 2. Mean Relative Quantity of Calcium Ions Released by Brain-Tissue Pairs as a Function of Exposure to 50-MHz Radiation Amplitude Modulated at 16 Hz

Power density (mW/cm^2)	Sham			Exposed			
	n	Mean	SE	n	Mean	SE	P
0.37	32	1.063	0.044	32	1.053	0.036	0.863
0.72	47	1.148	0.047	47	1.089	0.032	0.301
1.44	32	1.014	0.035	32	1.159	0.044	0.013
1.67	64	1.026	0.023	64	1.182	0.034	< 0.001
2.17	36	1.119	0.036	36	1.138	0.040	0.730
3.64	64	1.054	0.029	64	1.165	0.033	0.014
4.32	28	1.058	0.050	28	1.079	0.051	0.778

n = Number of samples.

DISCUSSION

In a previous report [Blackman et al, 1980] we described a range of power densities of 147-MHz carrier radiation, amplitude modulated at 9 or 16 Hz, which caused an alteration in calcium-ion efflux from brain tissue exposed in vitro. To explore further the conditions necessary to effect this radiation-induced change, we have investigated the effect of another carrier frequency, 50 MHz, for the power-density response of calcium-ion efflux.

The experimental design employed in this study was identical to the one used previously at 147 MHz [Blackman et al, 1980]. Brain tissue was treated with exposure to the radiation, and immediately afterwards another set of samples were treated under sham exposure. This alternation of exposure with sham exposure which occurred within one hour, minimized the influence of uncontrollable variables such as different hatching time of chickens, medium preparations, room temperature conditions, time of day, etc. Ten tubes, four containing brain tissue and six containing solution only, were treated at one time to take advantage of the broader power-density window observed previously when ten tubes were exposed together [Joines et al, 1980b]. We selected 16 Hz as the modulation frequency because a large amount of data from our previous studies would be available for comparison. Because previous research at 147 MHz demonstrated that the order of exposure and sham treatments did not affect the results, no additional work was done on

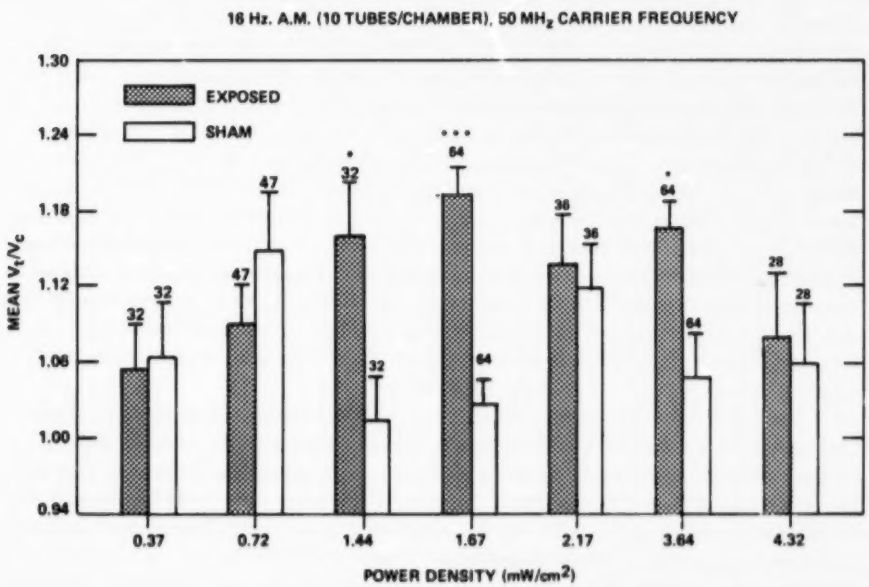

Fig. 1. Mean relative quantity (treated/control) of calcium ions released by chick-brain tissue exposed to 50-MHz radiation, sinusoidally amplitude modulated at 16 Hz, as a function of calculated power density. Four tubes containing brain tissue and six tubes containing only an equivalent amont of medium exposed simultaneously. The error bars represent one SE; the numbers above each bar indicate the number of samples. *, $P < 0.05$; ***, $P < 0.001$ – probability differences between exposed and sham groups, respectively.

order of treatment at 50 MHz. Our sham experiments, which gave an efflux ratio greater than 1.00, as we observed in our 147-MHz report, demonstrated that calcium-ion efflux was not identical in the treatment and control positions. We do not have an explanation for the cause of this difference. See the Discussion in Blackman et al [1980] for a treatment of the other variables.

Exposure of brain tissue in vitro to 50-MHz carrier radiation gave a power-density response that was markedly different from the results of an identical experiment performed with 147-MHz carrier radiation. Instead of a power-density window centered at 0.83 mW/cm^2, the results at 50 MHz indicated that there are at least two ranges of power densities that can induce enhanced calcium-ion efflux; one range spans 1.44 to 1.67 mW/cm^2 and is bounded by "no-enhancement" results at 0.72 and 2.17 mW/cm^2; the other range includes 3.64 mW/cm^2 and is bounded by 2.17 and 4.32 mW/cm^2. In a companion paper [Joines et al, 1980a], we described the physical and chemical considerations that are necessary to resolve the difference in effective power density observed using these two carrier frequencies.

The 50-MHz carrier radiation results also generally agree with the power-density window reported by Bawin et al [1979] using 450-MHz carrier radiation. They found enhanced efflux at 0.1 and 1.0 mW/cm^2, but not above or below these values. However, Bawin did not describe the exact sample positions and spacing, nor sample location with respect to the horn antenna used for irradiation. Thus, a detailed analysis of their results is not possible.

We have calculated the specific absorption rate (SAR) at the power density of 0.83 mW/cm^2 (for maximally enhanced calcium efflux at 147 MHz) to be 1.4 μW/g for the ten-sample case [Joines et al, 1980a]. By a similar calculation, the SAR corresponding to the highest power density for enhanced efflux at 50 MHz, 3.64 mW/cm^2, is 1.3 μW/g for the ten-sample case. These calculated SARs are essentially identical and are consistent with the SARs plotted in the Radiofrequency Radiation Dosimetry Handbook [Durney et al, 1978] for the smallest sphere-like object considered (pupae and quail egg). Thus, the energy dose for the 20-minute exposure is approximately 1.6 mJ/g. Even if there were no loss of thermal energy, the radiation-induced temperature change would be approximately 0.0004 °C in the exposed sample. In the generally accepted notion of thermal insult used in studies of RF radiation, changes in temperature of this magnitude are not considered to represent a major thermal insult to brain tissue. But if these SARs represent a thermal insult, the mechanism responsible must account for the lack of a detectable change in calcium-ion efflux at slightly higher SARs. While thermalization as a covariable cannot be ruled out, the evidence for a field-specific effect is strong.

Our research has shown that the efflux of calcium ions from brain tissue in vitro is associated with two variables: The frequency of modulation and the power density of incident radiation. Because the effective modulation frequencies are in the same range as the frequencies detected in electroencephalogram (EEG) recordings from live animals, this correlation provides a convenient basis for assuming that our results have some foundation in the neurophysiology of intact neural tissue. However, the finding that only power densities within a certain range can affect the efflux of calcium ions is difficult to explain.

The underlying mechanism responsible for the radiation-induced efflux of calcium ions may be based on a quantal response of the biological system [Pethig, 1979]. This could be experimentally tested by examining the reciprocity of dose rate and time of exposure. However, before the reciprocity experiments can be done, the time-rate-of-change of $^{45}Ca^{++}$ efflux must be characterized. In perfusion experiments with radiolabeled chick-brain tissue, we have observed two exponential processes contributing to the total

efflux. The relative contribution of these two processes to the total radiation-induced $^{45}Ca^{++}$ efflux is being assessed so that appropriate reciprocity experiments can be designed.

In this paper and its companion, we demonstrate that the difference in the effective power density at 147 and 50 MHz can be interpreted as dependent on the electric-field intensity within the sample. If the intensity of the electric field, or some correlate, is involved in the enhanced efflux of calcium ions, these results introduce a basis for inquiry, that coheres with principles of electromagnetic theory. These results confirm anew that efflux of calcium ions from in vitro brain tissue is affected by modulated RF radiation. The enhanced efflux occurs within at least two ranges of power density that are separated by a range of power densities that have no effect.

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REFERENCES

Ali JS (1975): A versatile temperature controlled exposure chamber for microwave bioeffects research. IEEE Trans Bio Eng BME-22:76–77.

Bawin SM, Sheppard AR, Adey WR (1977): Models of long-range order in cerebral macromolecules: Effects of ELF, VHF and UHF fields. Abstracts of scientific papers, 1977 International Symposium on the Biological Effects of Electromagnetic Waves, p 100. Washington DC: USNC-URSI, National Academy of Sciences.

Blackman CF, Elder JA, Weil CM, Benane SG, Eichinger DC, House DE (1979): Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effects of modulation frequency and field strength. Radio Sci 14(6S):93–98.

Blackman CF, Benane SG, Elder JA, House DE, Lampe JA, Faulk JM (1980): Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effect of sample number and modulation frequency on the power-density window. Bioelectromagnetics 1:35–43.

Durney CH, Johnson CC, Barber PW, Massondi H, Iskander MF, Lords JL, Ryser DK, Allen SJ, Mitchell JC (1978): "Radiofrequency Radiation Dosimetry Handbook," Ed 2. Report SAM-TR-78-22, USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks AFB, TX. Springfield, Virginia: NTIS.

Joines WT, Blackman CF (1980a): Power density, field intensity, and carrier frequency determinants of RF-energy–induced calcium-ion efflux from brain tissue. Bioelectromagnetics (this issue).

Joines WT, Blackman CF, Hollis MA (1980b): Broadening of the RF Power-Density Window for Calcium-ion Efflux from Brain Tissue, IEEE Trans BioMed Eng (submitted).

Pethig R (1979): "Dielectric and Electronic Properties of Biological Materials." New York: John Wiley & Sons.

Weil CM (1978): The characteristic impedance of rectangular transmission lines with thin center conductor and air dielectric. IEEE Trans Microwave Theory Tech MTT-26:238–242.